GC-MS ANALYSIS OF ETHANOL EXTRACT OF *SARCOSTEMMA SECAMONE* (L) BENNET (ASCLEPIADACEAE)

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ABSTRACT

The investigation was carried out to determine the possible bioactive components of whole plant of *Sarcostemma secamone* using GC-MS analysis. The chemical compositions of the ethanol extract of whole plant of *Sarcostemma secamone* was investigated using Perkin – Elmer Gas Chromatography – Mass Spectrometry, while the mass spectra of the compounds found in the extract was matched with the National Institute of Standard and Technology (NIST) library. Fourteen compounds were identified; Dihydrotachysterol (22.78%) was found to be major component followed by Methane, nitro (17.72%), Butanoic acid, 3,7-Dimethyl-6-octeny ester (11.39%), Phytol (10.76%), Squalene (10.13%), 9,12-Octadecadienoic acid (Z,Z)-, phenylmethyl ester (6.96%), 9-Octadecenoic acid (Z)-, phenylmethyl ester (6.96%) and Didodecyl phthalate (3.16%).

Key words: Ethnomedicine; *Sarcostemma Secamone*, GC-MS, Arthritis.

INTRODUCTION

Sarcostemma secamone (L) Bennet, is an important medicinal plant belonging to the family Asclepiadaceae. It is used in the traditional systems of medicine for various ailments. The decoction of the plant is useful to gargle for throat and mouth infection. The latex is bitter and used as a vulnerary. Fresh roots are prescribed for jaundice (Chopra *et al*., 1956, Chopra *et al*., 1958, Anonymous, 1966; Nadkarni, 1982). The milky sap forms a wash for ulcers. In combination with turpentine, it is prescribed for itch (Kirtikar and Basu 1976). The plant is hot, bitter, tonic, expectorant, pungent, dry and indigestible causes flatulence, diuretic, laxative, aphrodisiac, anthelmintic, useful in leucoderma and bronchitis. The juice is used in gleet, gonorrhea, pain in the muscles, cough and given to children as an astringent (Poornima *et al*., 2009). Leaf powder stimulates arculatory system, increases secretion of urine and activates uterus (Prajapati *et al*., 2003). The fruit juice is used in gonorrhoea and pain in muscles (Kirtikar and Basu, 1976). The leaves, roots and latex of *Sarcostemma secamone* are employed in treating many diseases like mouth ulcer, sour throat, jaundice and ulcers (Khan, 2002, Satyavathi *et al*., 1987, Jain, 1991). Hence, the objective of the present study is to identify the phytochemical constituents with the aid of GC-MS technique.

MATERIALS AND METHODS

The well grown and healthy whole plant of *Sarcostemma secamone* (L.) Bennet were collected from natural forests of Western Ghats at Thanniparai, Srivilliputhur, Virudhunagar District, Tamil Nadu. The plant samples were cleaned, shade dried and pulverized to powder in a mechanical grinder. Required quantity of powder was weighed and transferred to stoppered flask, and treated with ethanol until the powder is fully immersed. The flask was shaken every hour for the firsts 6 hours and then it was kept aside and again shaken after 24 hours. This process was repeated for 3 days and then the extract was filtered. The extract was collected and evaporated to dryness by using vacuum distillation unit. The final residue thus obtained was then subjected to GC – MS analysis.

GC-MS Analysis

GC-MS analysis of these extracts performed with GC clarus 500 Perkin Elmer system and Gas chromatograph interfaced to a Mass spectrometer (GC-MS) equipped with a Elite – 1 fused silica capillary column (30 mm x 0.25 mm 1D x 1 um df, composed of 100% Dimethyl poly siloxane). For GC-MS detection, and electron ionization system with ionizing energy of 70 eV was used.

Helium gas (99.999%) was used as the carrier gas at constant flow rate 1 ml / min and an injection volume of 2 ul was employed (Split ratio of 10:1); Injector temperature 250^0C; ion-source temperature 280^0C. The oven temperature was programmed from 110^0 C (isothermal for 2 min) with an increase of 10^0C/min, to 200^0C, then 5^0C/min to 280^0C, ending with a 9 min isothermal at 280^0C. Mass spectra were taken at 70 eV, a scan interval of 0.5 seconds and fragments from 45 to 450 Da. Total GC running time was 36 minutes. The relative % amount of each component was calculated by comparing its average peak area to the total areas, software adopted to handle mass spectra and chromatograms was a turbomass.

Interpretation on mass spectrum of GC-MS was done using the database of National Institute of Standard and Technology (NIST) having more than 62,000 patterns. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

RESULTS AND DISCUSSION

The components present in the ethanol extract of whole plant of *Sarcostemma secamone* were identified by GC-MS analysis (Figure 1). The active principles with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) in the ethanol extract of whole plant of *Sarcostemma secamone* are presented in the Table 1. Fourteen compounds were detected in ethanol extract of the whole plant. The results revealed that, Dihydrotachysterol (22.78%) was found as major component followed by Methane, nitro (17.72%), Butanoic acid, 3,7-dimethyl-6-octenyl ester (11.39%), Phytol (10.76%), Squalene (10.13%), 9,12-Octadecadienoic acid (Z,Z)-, phenylmethyl ester (6.96%), 9-Octadecenoic acid (Z)-, Phenyl methyl ester (6.96%) and Didodecyl phthalate (3.16%). Figure 2, 3, 4 and 5 shows the mass spectrum and structure of 9,12-Octadecadienoic acid (Z,Z)- phenylmethyl ester, Didodecyl phthalate, Squalene and Dihydrotachysterol respectively. Table 2 lists the major phytocomponents and their biological activities obtained through the GC – MS study of *Sarcostemma secamone*.

Table 1: Components detected in the whole plant of ethanol extract of *Sarcostemma Secamone*

No.	RT	Name of the compound	Molecular Formula	MW	Peak Area %
1.	2.32	Methane, nitro-	CH_3NO_2	61	17.72
2.	11.86	Butanoic acid, 3,7-dimethyl-6-octenyl ester	$C_{14}H_{26}O_2$	226	11.39
3.	13.34	Dibutyl phthalate	$C_{16}H_{22}O_4$	278	2.53
4.	13.73	Heptanoic acid, 2-ethyl-	$C_9H_{18}O_2$	158	1.27
5.	15.28	Phytol	$C_{20}H_{40}O$	296	10.76
6.	15.95	1-Tetradecyne	$C_{14}H_{26}$	194	1.90
7.	16.04	10-Undecyn-1-ol	$C_{11}H_{20}O$	168	2.53
8.	19.78	Hexadecanal	$C_{16}H_{32}O$	240	0.63
9.	21.25	Didodecyl phthalate	$C_{32}H_{54}O_4$	502	3.16
10.	23.57	2,3-Anhydro-d-galactosan	$C_6H_8O_4$	144	1.27
11.	25.13	Squalene	$C_{30}H_{50}$	410	10.13
12.	31.35	9,12-Octadecadienoic acid (Z,Z)-, phenylmethyl ester	$C_{25}H_{38}O_2$	370	6.96
13.	31.88	9-Octadecenoic acid (Z)-, phenylmethyl ester	$C_{25}H_{40}O_2$	372	6.96
14.	33.27	Dihydrotachysterol	$C_{28}H_{46}O$	398	22.78

Table 2: Activity of Phyto-Components identified in the ethanol extracts of the whole plant of *Sarcostemma Secamone*

No.	Name of the compound	Molecular Formula	Nature of compound	**Activity
1.	Methane, nitro-	CH_3NO_2	Nitrogen	Antimicrobial
2.	Butanoic acid, 3,7-dimethyl-6-octenyl ester	$C_{14}H_{26}O_2$	Ester	Antimicrobial
3.	Dibutyl phthalate	$C_{16}H_{22}O_4$	Plasticizer	Antimicrobial Anti-fouling
4.	Phytol	$C_{20}H_{40}O$	Diterpene	Antimicrobial Anti-inflammatory Anticancer Diuretic
5.	10-Undecyn-1-ol	$C_{11}H_{20}O$	Alcoholic	Antimicrobial
6.	Hexadecanal	$C_{16}H_{32}O$	Aldehyde	Antimicrobial
7.	Didodecyl phthalate	$C_{32}H_{54}O_4$	Plasticizer	Antimicrobial Antifouling
8.	2,3-Anhydro-d-galactosan	$C_6H_8O_4$	Sugar moiety	Preservative
9.	Squalene	$C_{30}H_{50}$	Triterpene	Anticancer Antimicrobial Antioxidant Chemo preventive Pesticide
10.	9,12-Octadecadienoic acid (Z,Z)-, phenylmethyl ester	$C_{25}H_{38}O_2$	Linoleic acid ester	Anti- tumor Sunscreen Hypocholesterolemic Nematicide Antiarthritic Hepatoprotective Anti androgenic Hypocholesterolemic Nematicide 5-Alpha reductase inhibitor
11.	9-Octadecenoic acid (Z)-, phenylmethyl ester	$C_{25}H_{40}O_2$	Oleic acid ester	Antihistaminic Anticoronary Insectifuge Antieczemic Antiacne Anti-inflammatory, Antiandrogenic Cancer preventive, Dermatitigenic Hypocholesterolemic, 5-Alpha reductase inhibitor, Anemiagenic Insectifuge, Flavor
12.	Dihydrotachysterol	$C_{28}H_{46}O$	Steroid compound	Antimicrobial Antiinflammatory Anticancer Diuretic Antiasthma Antiarthritic

***Source: Dr.Duke's: Phytochemical and Ethnobotanical Database.**

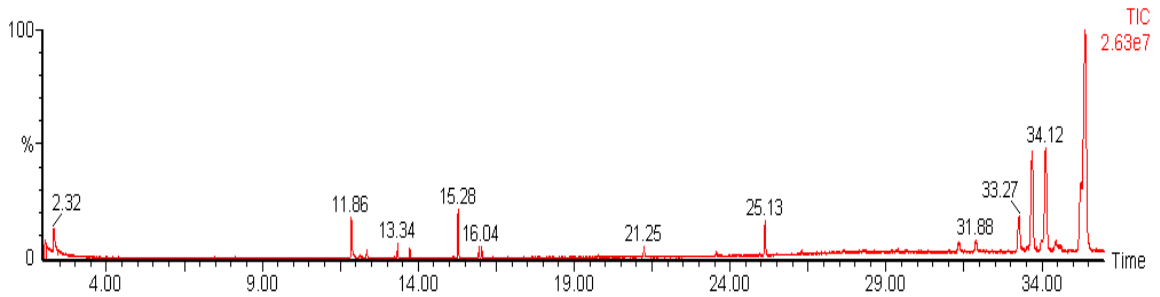

Fig 1: GC-MS Chromatogram of the ethanol extracts of whole plant of *Sarcostemma Secamone*

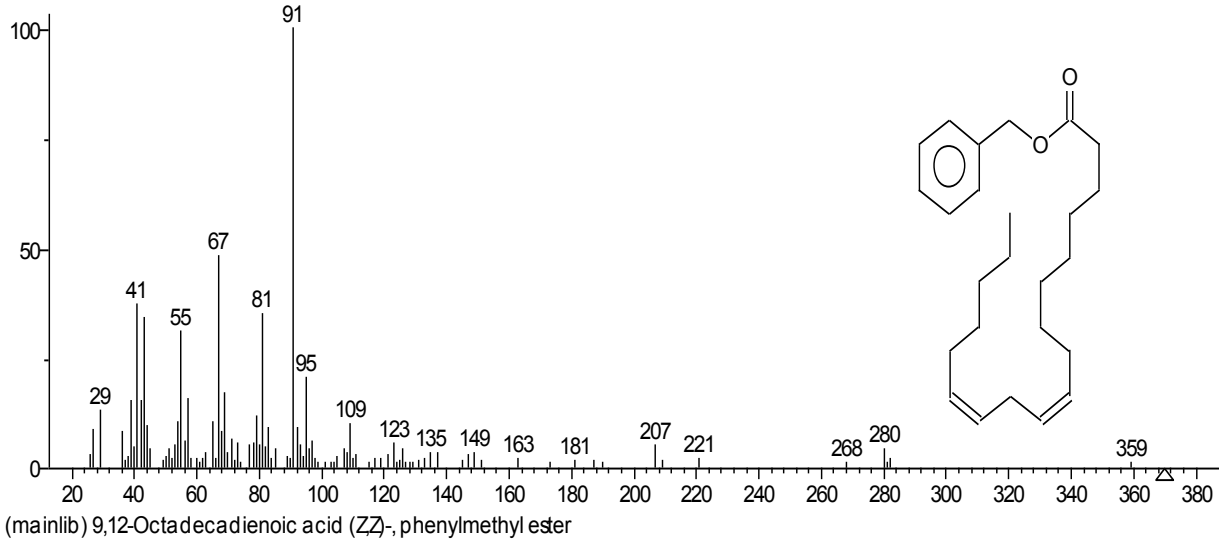

Fig 2: Mass spectrum of 9,12-Octadecadienoic acid (Z,Z)-, phenylmethyl ester

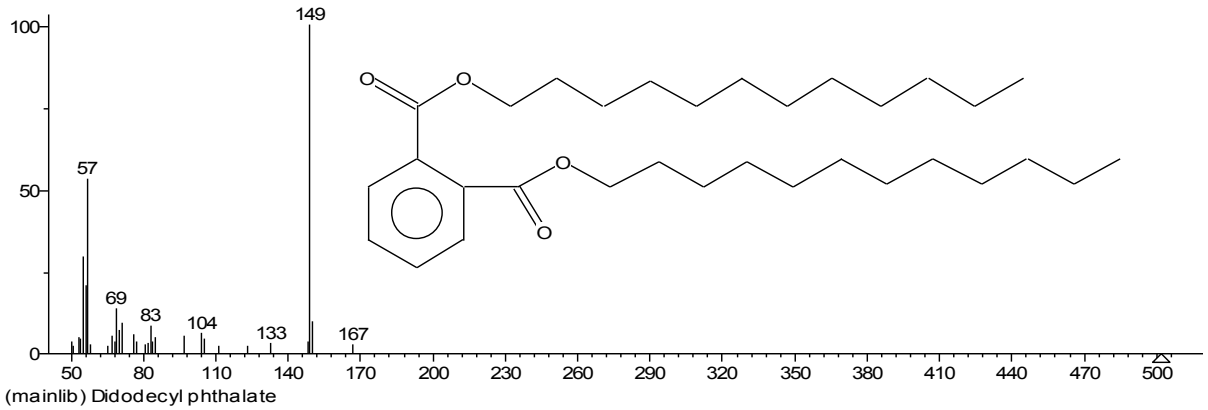

Fig 3: Mass spectrum of Didodecyl phthalate

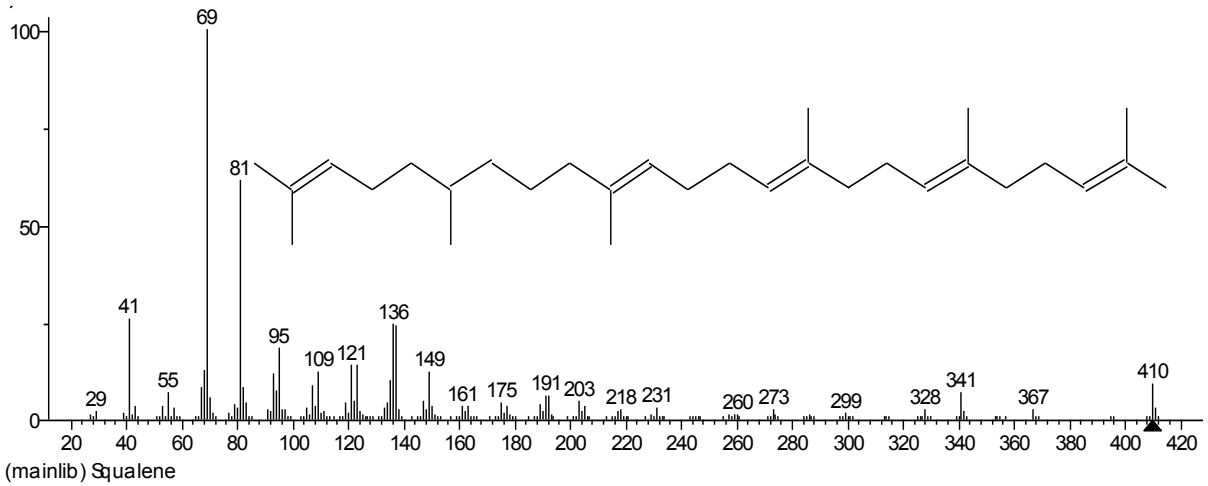

Fig 4: Mass spectrum of Squalene

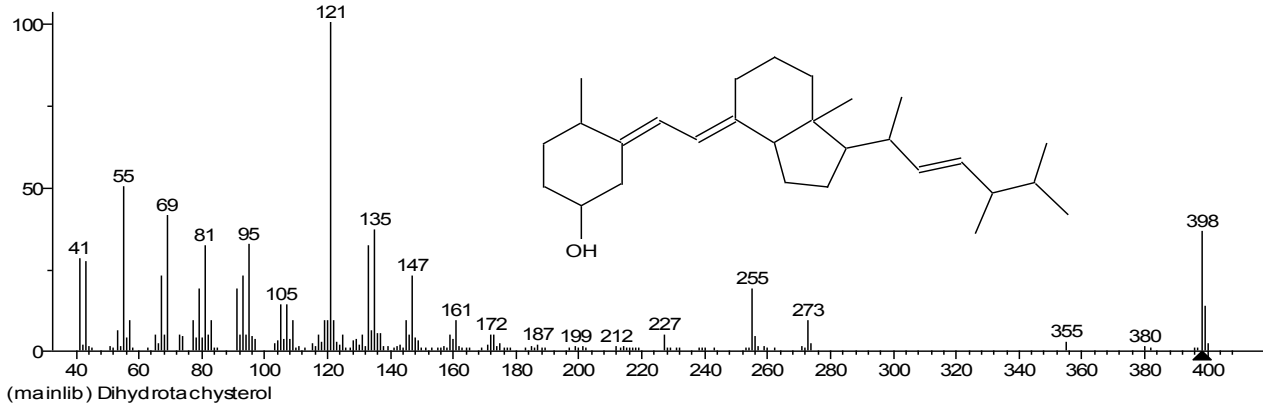

Fig 5: Mass spectrum of Dihydrotachysterol

Among the identified phytochemicals, squalene has antioxidant activity. Recently it has been found that, squalene possesses chemo-preventive activity against the colon carcinogenesis (Rao *et al*., 1998). Phytol, a bioactive principle, detected from *Sarcostemma secamone* (L.) Bennet is also found to be effective at different stages of arthritis. It is found to give good as well as preventive and therapeutic results against arthritis. The results show that, reactive oxygen species – promoting substances such as phytol constitute a promising novel class of pharmaceuticals for the treatment of rheumatoid arthritis and possibly other chronic inflammatory diseases (Ogunlesi *et al*., 2009). Thus, this type of GC – MS analysis is the first step towards understanding the nature of active principles in this medicinal plants and this type of study will be helpful for further detailed study.

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